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Characterisation Of Small Molecule Activators Of GPR43 Using The NCI-H716 Enteroendocrine Cell Line

GPR43 is a short chain fatty acid receptor that is expressed in a range of cell types, including adipocytes, immune cells, pancreatic islets and intestinal epithelial cells (1). The receptor is involved in the regulation of intestinal homeostasis and inflammation, appetite and insulin secretion and as such, GPR43 agonists/positive allosteric modulators (PAMs) may be of therapeutic value in metabolic (e.g. diabetes) and inflammatory disorders (e.g. colitis) (1). GPR43 has been shown to couple to $G\alpha_i/\alpha_q$ *in vitro*, however the contribution of these signalling pathways to the *in vivo* effects of GPR43 activation is unclear.

The NCI-H716 cell line, in addition to a recombinant CHO line expressing human GPR43, was used to profile two synthetic GPR43 activators, an Amgen allosteric agonist (phenylacetamide 2 (2); PC2) and an Euroscreen agonist (compound 1 (3); ES1). NCI-H716 is a human cecal carcinoma cell line, often referred to as an intestinal enteroendocrine cell model (4), which endogenously expresses GPR43. In this work, GPR43 engagement of $G\alpha_i$ and $G\alpha_q$ pathways was examined using an HTRF cAMP and fluorescent calcium (Ca^{2+}) flux assay respectively, in agonist and PAM modes (with EC_{20} sodium acetate).

Full agonism was observed for ES1 in both assays and cell lines, with no PAM activity detected. In calcium and cAMP assays, ES1 was similarly potent at the recombinant and native receptor, however a reduction in potency (6 - 9 fold) was observed in the calcium versus cAMP assays (EC_{50} values (μM): NCI-H716 Ca^{2+} 0.76, cAMP 0.12; CHO-GPR43 Ca^{2+} 0.36, cAMP 0.041). PC2 also showed full agonism with no PAM activity in the cAMP assay, however higher potency agonism (8 fold) was detected in the CHO-GPR43 cells compared to the NCI-H716 line (cAMP EC_{50} values (μM): NCI-H716 0.16, CHO-GPR43 0.02). In calcium flux, PC2 was a low potency (EC_{50} 2.4 μM; 120 fold less potent than in cAMP), full agonist in the recombinant cell line and although some agonism was observed in NCI-H716 cells, this was only at the highest concentrations tested. PC2 showed potent PAM activity in both cell lines in the flux assay (EC_{50} values (μM): NCI-H716 0.02, CHO-GPR43 0.006), in clear contrast to the cAMP assay.

Using both native and recombinant cell systems, this work highlighted distinct *in vitro* pharmacological profiles for ES1 and PC2, with ES1 being a $G\alpha_i/\alpha_q$ agonist and PC2 being a $G\alpha_i$ agonist, weak $G\alpha_q$ agonist and $G\alpha_q$ PAM. Both compounds showed a similar profile in GPR43 recombinant and native cell lines, and although this provides some confidence as to their mechanism of action, further investigation *in vivo* would be needed to confirm this and help define the nature of GPR43 modulation that will be most therapeutically effective.

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